PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :
A61K 39/385, 39/21, 39/12
A61K 47/48, C07K 17/02, 7/02
C07K 7/06, 7/08, 7/10

A1

(11) International Publication Number: WO 93/03766

(43) International Publication Date: 4 March 1993 (04.03.93)

(21) International Application Number: PCT/US92/06688

(22) International Filing Date: 11 August 1992 (11.08.92)

(30) Priority data:
744,281 13 August 1991 (13.08.91) US

(71) Applicants: REPLIGEN CORPORATION [US/US]; One Kendall Square, Cambridge, MA 02139 (US). THE ROCKEFELLER UNIVERSITY [US/US]; 1230 York Avenue, New York, NY 10021 (US).

(72) Inventors: TAM, James, P. ; 500 East 63rd Street, New York, NY 10021 (US). PROFY, Albert, T. ; 28 Essex Street, Cambridge, MA 02139 (US).

(74) Agent: CLARK, Paul, T.; Fish & Richardson, 225 Franklin Street, Boston, MA 02110 (US).

(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE).

Published
With international search report.

(54) Title: MULTIPLE ANTIGEN PEPTIDES FOR USE AS HIV VACCINES

(57) Abstract

In general, the invention features a multiple antigenic peptide system including a dentritic core and a peptide, wherein the peptide includes the sequence IGPGR (SEQ ID NO: 3), and the multiple antigen peptide system, when injected into a mammal, is capable of eliciting an immune response.

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MULTIPLE ANTIGEN PEPTIDES FOR USE AS HIV VACCINES

Background of the Invention

The field of the invention is vaccines for prevention and treatment of HIV infection.

Highly specific and immunogenic antigens are preferred as vaccines. While the immunogenicity of an antigen can be increased by coupling a protein carrier to the antigen, this approach has several drawbacks. First, if the carrier is large, significant humoral immune response can be directed against the carrier rather than the antigen. Second, a large carrier can suppress humoral response to the antigen. Finally, the coupling of an antigen to a protein carrier can alter the immunogenic determinants of the antigen.

Multiple antigen peptide systems (MAPS) are designed to overcome the problems observed with conventional protein carriers. Most MAPS are composed of several peptide antigens covalently linked to a branching, dendritic core composed of bifunctional units (e.g., lysines). Thus, a cluster of antigenic epitopes form the surface of a MAPS and a small matrix forms its core. As a result, the core is not immunogenic. MAPS have been used to prepare experimental vaccines against hepatitis (Tam et al., *Proc. Natl. Acad. Sci. USA* 86:9084, 1989), malaria (Tam et al., *J. Exp. Med.* 171:299, 1990), and foot-and-mouth disease. A further advantage of MAPS is that they are chemically unambiguous. This allows different epitopes, such as B cell and T cell epitopes, to be arranged and a particular arrangement and stoichiometry.

European Patent Application 89200145.4 describes a process for preparing MAPS by reacting a branched

structure based on an amino acid such as lysine with a separately synthesized antigenic compound.

European Patent Application 89301288.0 describes peptides (CRIKQIINMWQEVGKAMYAPPISGQIRC (SEQ ID NO: 1), QSVEINCRTPNNNTRKSIRIQRGPGRAFVTIGK (SEQ ID NO: 2), and analogs thereof) which are specifically immunoreactive with antibodies to HIV and suggests that MAPS which include these peptides can be used for immunization to prevent HIV infection.

Hart et al. (*J. Immunol.*, 145:2677, 1990) report that a synthetic peptide construct which includes amino acids 428-443 and 303-321 of HIV-I-III_B envelope protein gp120, when used as a carrier-free immunogen in primates, can induce a high titer of neutralizing anti-HIV antibodies and can induce T cell proliferative response against native HIV-I gp120.

Palker et al. (*Immunology* 142:3612, 1989) describes the use of a 16 amino acid T cell epitope from HIV-I-III_B fused to a synthetic peptide which includes a type-specific neutralizing determinant of a particular HIV-I strain (III_B, MN or RF) to immunize goats. Both T cells and B cells responded to epitopes within the type-specific neutralizing determinant.

PCT Application PCT/US90/02039 discloses multiple antigen peptide systems in which a large number of each of T cell and B cell malarial antigens are bound to the functional groups of a dendritic core molecule.

Summary of the Invention

In general, the invention features a multiple antigenic peptide system including a dendritic core and a peptide, wherein the peptide includes the sequence IGPGR (SEQ ID NO: 3), and the multiple antigen peptide system, when injected into a mammal, is capable of eliciting an immune response.

In a preferred embodiment, the peptide includes a pair of six amino acid sequences flanking the sequence IGPGR (SEQ ID NO: 3), the flanking sequences taken together having at least 36% homology to the pair of six amino acid sequences flanking the sequence IGPGR within the V3 loop of HIV-I-MN prototype virus.

The V3 loop sequence of the gp120 envelope protein of HIV-I-MN includes the 35 amino acids of HIV-I-MN from the invariant cysteine at position 303 to the invariant cysteine at position 3-8, inclusive. The HIV-I-MN prototype virus is defined by a particular amino acid subsequence within the V3 loop region of the gp120 envelope protein having the sequence KRKRIHIGPGRAFYTTK (SEQ ID NO: 4). (Amino acid sequences are presented in the standard single-letter code throughout.)

In another preferred embodiment the peptide includes the sequence KRKRIHIGPGRAFYTTK (SEQ ID NO: 4).

In a preferred embodiment, the multiple antigenic peptide system includes a T cell epitope. In more preferred embodiments, the T cell epitope is covalently linked in tandem to the peptide; the T cell epitope includes the sequence QIINMWQEVGKAMYA (SEQ ID NO: 5). By "T cell epitope" is meant a peptide capable of eliciting a proliferative T cell response. Preferably, the T cell epitope is at least seven amino acids long.

In other preferred embodiments, the dendritic core includes lysine; the dendritic core is tetravalent.

In another preferred embodiment, the peptide is between 10 and 40 amino acids long.

In other preferred embodiments, the peptide includes the sequence HIGPGR (SEQ ID NO: 6); the peptide includes the sequence IHIGPGR (SEQ ID NO: 7), the peptide includes the sequence RIHIGPGR (SEQ ID NO: 8); the peptide includes the sequence IGPGRA (SEQ ID NO: 9); the peptide includes the sequence IGPGRAF (SEQ ID NO: 10);

the peptide includes the sequence KRKRIHIGPGRAFYTTKN (SEQ ID NO. 11).

In a related aspect, the invention features a method of immunizing a mammal to inhibit HIV infection. The method includes administering to the mammal the multiple antigen peptide system described above.

In related aspect, the invention features a method for eliciting an immune response against HIV in a mammal. The method includes administering to the mammal the multiple antigen peptide system described above.

In another related aspect, the invention features a vaccine which includes an immunologically effective amount of the multiple antigen peptide system described above.

In yet another related aspect, the invention features a method for generating antibodies. The method includes administering to a mammal an antibody-generating amount of the multiple antigen peptide system described above.

Multiple antigen peptide system (MAPS) is the commonly used name for a molecule composed of two or more, usually identical, antigenic molecules covalently attached to a dendritic core which is composed of bifunctional units. The dendritic core molecule is a branching molecule in which a first bifunctional unit is linked to two additional bifunctional units each of which may be attached to two additional bifunctional units to form a third generation molecule. This pattern may be repeated any number of times to form higher generation molecules. For each molecule the number of free functional groups is equal to 2^n, where n is equal to the generation of the molecule. A third generation molecule thus has 8 free functional groups which can be attached to 8 peptides.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Detailed Description

The drawings are first briefly described.

Figure 1 is a schematic representation of several tetravalent MAPS and the sequences of the peptides attached to the lysine core. T cell epitopes are represented by the shaded rectangles in the schematic drawings; B cell epitopes are represented by unshaded rectangles. The amino acid sequences of peptides B1-B9 and T are listed.

Figure 2 is a pair of graphs which depict the results of ELISA assays used to measure mouse antisera binding to HIV-I-III$_B$ peptides (panel A) and HIV-I-III$_B$ gp120 (panel B). Antisera were raised using B1 peptide (filled circles), B2 peptide (open circles), and B3 peptide (filled squares). Antisera, serially diluted, were tested for their binding activity to wells coated either with 5 μg of the same peptide used to raise the antisera (panel A) or 0.1 μg of recombinant gp120 (panel B). Goat anti-mouse IgG was used as secondary antibody. The mean absorbance (405 nm) is plotted as a function of the reciprocal dilution of antisera.

Figure 3 is a graph which depicts the results of ELISA assays used to measure mouse antisera binding to peptide B4T. Antisera were obtained after three intraperitoneal immunizations with MAP-B4T (solid circles) or B4T peptide (open squares). The mean absorbance (405 nm) is plotted as a function of the reciprocal dilution of antisera.

HIV-I-MN and HIV-I-MN Viral Variant Peptides in MAPS

In the experiments described below MAPS which include peptides derived from the V3 loop of the HIV-I external envelope protein (gp120) were used to raise antisera in mice, rabbits and guinea pigs. The V3 loop of gp120 includes all of the amino acids from cysteine 303 to cysteine 338 of HIV-I. In intact gp120 a disulfide bond between these two cysteines forms a loop (V3 loop). The V3 loop represents one of the most variable regions of the envelope protein. Among various HIV-I isolates the sequence of the V3 loop varies by as much as 50%. Despite this variability a relatively conserved GPGR sequence lies at the tip of the loop. This is flanked on both sides by more variable strain-specific sequences. Analysis of the amino acid sequences of the V3 loop of 245 different HIV-I isolates revealed that the V3 loop sequence of HIV-I-MN differs from the consensus at only 6 of 35 amino acid positions (La Rosa et al. *Science* 249:932, 1990).

Multiple antigen peptide system (MAPS) is the commonly used name for a combination antigen/antigen carrier that is composed of two or more, usually identical, antigenic molecules covalently attached to a dendritic core which is composed of bifunctional units. The dendritic core of a multiple antigen peptide system can be composed of lysine molecules. For example, a lysine is attached via peptide bonds through each of its amino groups to two additional lysines. This second generation molecule has four free amino groups each of which can be covalently linked to an additional lysine to form a third generation molecule with eight free amino groups. A peptide may be attached to each of these free groups to form an octavalent multiple peptide antigen. Alternatively, the second generation molecule having four free amino groups can be used to form a tetravalent MAPS, i.e., a MAPS having four peptides covalently linked to

the core. Many other molecules, including aspartic acid and glutamic acid, can be used to form the dendritic core of a multiple peptide antigen system. The dendritic core, and the entire MAPS may be conveniently synthesized on a solid resin using the classic Merrifield synthesis procedure.

Multiple antigen peptide systems have many advantages as antigen carrier systems. Their exact structure and composition is known; the ratio of antigen to carrier is quite high; and several different antigens, e.g., a B cell epitope and a T cell epitope, may be attached to a single dendritic core. When both a B cell epitope and a T cell epitope are present it is preferable that they are linked in tandem on the same functional group of the dendritic core. Alternatively the T cell epitope and the B cell epitope may be on separate branches of the dendritic core. Preferably, the T cell epitope is a helper T cell epitope; however a cytotoxic T cell epitope may also be used. Useful T cell epitopes may be derived from the HIV-I envelope protein. However, it is not necessary that the B cell epitope and the T cell epitope both be derived from the HIV-I gp120 envelope protein. T cell epitopes from different HIV-I proteins (e.g., those encoded by the *nef*, *gag*, *tat*, *rev*, *vif*, *pol*, *vpr*, *vpu*, or *vpx* genes), different retrovirus, or unrelated organisms (e.g., malarial antigens or tetanus toxoid) may be used. T cell epitopes can be identified by a T cell proliferation assay (described herein below).

Multiple antigen peptide systems and methods for their preparation are described more fully in PCT Application WO 90/11778, and European Patent Application 89200145.4 both of which are hereby incorporated by reference.

In the experiments described herein MAPS that include peptides derived from the V3 loop of the gp120 protein of HIV-I-MN are shown to raise potent antisera. Accordingly, vaccines which employ HIV-I-MN MAPS are expected to be particularly useful for generating an immune response in humans.

Also described below are experiments which demonstrate that the addition of a T cell epitope often increases the immunogenicity of HIV-I MAPS.

Peptides derived from the V3 loop of HIV-I-MN are capable of raising broadly neutralizing antibodies. Such antibodies can block infection of cultured cells by a wide range of HIV-I strains (PCT Patent Application PCT/US90/03157, hereby incorporated by reference). Accordingly, MAPS which employ peptides derived from the V3 loop of HIV-I-MN are expected to generate similarly broadly neutralizing antibodies.

Experimental Procedures

The experiments described herein were performed according to the procedures described below.

Animals Outbred CD-1 mice and New Zealand White rabbits were purchased from Charles River Laboratories (Wilmington, MA). Outbred Dunkin-Hartley guinea pigs were raised and immunized by Hazelton Biotechnologies Company (Denver, PA).

Synthesis of peptides Synthetic peptides were prepared manually by a stepwise solid-phase peptide synthesis (Tam, *Proc. Nat'l. Acad. Sci. USA* 85:5409, 1988; Merrifield, *Science* 232:341, 1986) on t-butoxycarbonyl (Boc)-Ala-OCH_2-Pam resin (Mitchell et al., *J. Am. Chem. Soc.* 98:7357) or p-alkoxybenzyl alcohol resin. The mono-epitope peptides were synthesized by Boc-benzyl chemistry. The di-epitope peptides were synthesized by Fmoc-tertbutyl chemistry. The coupling was mediated with DCC/1-hydroxybenzotriazole in dimethylformamide. After

completion of synthesis, each MAP-resin was treated with a deprotecting reagent to remove N-protecting groups and cleaved with low-high hydrofloride (Boc-chemistry; Tam et al., *J. Am Chem. Soc.* 105:6441, 1983) or 95% trifluoroacetic acid (Fmoc-chemistry). The peptide was extracted in 8M urea in 0.1M Tris-HCl, pH 8, then dialyzed several times and lyophilized. All MAPS gave satisfactory amino acid analysis.

Preparation of MAPS The preparation of MAPS is described by Posnett et al. (*J. Biol. Chem.* 263:17179, 1988) and Tam et al. (*Proc. Nat'l. Acad. Sci. USA* 85:8409, 1988). The process essentially employs conventional solid-phase peptide synthesis as described by Merrifield (*J. Am. Chem. Soc.* 85:2149, 1963). Briefly, a resin coupled to a t-butoxycarbonyl-substituted (Boc-substituted) amino acid is reacted with 50% trifluoroacetic acid to remove Boc and the resulting salt is neutralized with diisopropoylethylamine. The first part of the lysine core is then added by reacting the amino acid-coupled resin with Boc-Lys(Boc) in dimethylformamide followed by reaction with dicyclohexylcarbodiimide and CH_2Cl_2. A second such synthetic cycle yields a branched tetrapeptide on which four peptides may be synthesized using conventional solid-phase synthesis techniques. It is also possible to separately synthesize the lysine core and the peptides and then couple the peptides to the core in a subsequent step (European Patent Application 89200145.4).

It may be desirable to use peptides which have been circularized prior to attachment to the dendritic backbone. This circularization can be accomplished via disulfide bond formation between cysteines present in the peptide. Such an arrangement is particularly desirable for peptides derived from the V3 loop of gp120 since the sequence in this region forms a loop in the intact virus.

Immunization procedure The animals were injected four times, at two week intervals, using complete Freund's adjuvant (Sigma, St. Louis, MO) for the first injection, and incomplete Freund's adjuvant for the booster injections. Mice (five for each peptide) received intraperitoneally 50 μg of the peptide for each injection. Guinea pigs (three for each group) received subcutaneously 100 μg of the peptides the first and second injections and 50 μg for the last two injections. Rabbits (two for each peptide) were injected intradermally with 400 μg (first injection, day 0) and 200 μg (second injection, day 14) and subsequently intramuscularly with 200 μg of the peptides at day 28 and day 42. The animals were bled immediately before each injection. The antisera used for the reported experiments were obtained fifteen days after the last injection.

ELISA Assays Mouse and rabbit antisera were analyzed by standard direct ELISA using flat-bottomed microplates (Maxisorp, Nunc, Denmark) coated with 5 μg/well of each peptide or 0.1 μg/well of purified recombinant gp120 (Repligen, Cambridge, MA). The assays of the guinea pig sera were performed using plates coated with the 24 amino acid peptides, RP135 (III_B), RP139 (RF), and RP142 (MN) (Rusche et al., *Proc. Nat'l Acad Sci. USA* 85:3198, 1988). The plates were blocked for 90 min at 37°C with the diluent buffer (PBS + 1% calf serum). Incubation with antisera, serially diluted in the same buffer, was carried out for 2 hr at 37°C and was followed by three washes with 0.05% Tween 20 in PBS. Phosphatase-conjugated goat secondary antibody (Sigma), diluted 1:1000, was then added for 2 hr at 37°C. After an additional three washes, the substrate p-nitrophenyl phosphate (1 mg/ml; Sigma) in diethanolamine buffer (pH 9.8) was added, and the bound secondary antibody was

detected at 405 nm. The antibody titer was calculated as the reciprocal of the antiserum dilution giving the half maximal response. The optical density obtained with preimmunization sera was always less than 0.1 units.

The recombinant virus expressing the env gene is used to infect CD4+ cells (e.g., CEM, MOLT4, or SUP-T1 cells). The HIV envelope protein presented on the surface of these cells will bind to the cell surface receptor, CD4, resulting in the fusion of the cells and the formation of giant multinucleated cells called syncytia. Syncytium formation can be assayed in the presence or absence of antiserum at a series of dilutions. The number of syncytia that are formed are quantified at an appropriate time post-infection. The preferred MAPS are those which raise antisera that inhibits syncytia formation even when the antisera is substantially diluted.

In the experiments described below CD4-positive CEM cells (Accession Number CCL119, American Type Culture Collection, Rockville MD) were infected with recombinant vaccinia viruses expressing full length gp160 at a multiplicity of infection of 1. For HIV-I-III_B and HIV-I-RF, the recombinant virus expressed the entire envelope gene. For HIV-I-MN the recombinant virus expressed the V3 region of HIV-I-MN inserted into the gp160 of HIV-I-III_B as described by Scott et al. (*Proc. Nat'l. Acad. Sci. USA* 87:8597, 1990). Immune sera were added to the cultures 1 hr post-infection and syncytia were counted 24 hr post-infection. The fusion inhibition titer for each immune serum is defined as the reciprocal of the dilution which reduces the number of syncytia to 10% of the number observed in the presence of a normal serum control.

Antisera can also be assessed using a viral neutralization assay. In this assay viral reverse

transcriptase activity is used as a measure of viral activity. Dilutions of antiserum are incubated with HIV and are than added to HIV susceptible CD4+ cells. Such an assay is described by Robey et al., *Proc. Nat'l. Acad. Sci. USA* 83:7023, 1986; Popovic et al., *Science* 224:497, 1984; and Robert-Guroff, *Nature* 316:72, 1985).

PEPSCAN Antigen domains can be identified by pepscan analysis as described by Geyson et al. (*J. Immunol. Methods* 102:259, 1987). Briefly, overlapping peptides derived from the sequence of a peptide to be analyzed can be synthesized on the tips of polyethylene rods. The rods are then assembled into a holder with the format of a microtiter plate. All the subsequent reactions can be carried out at the tips of the rods using a microtiter plate. Nonspecific binding can be avoided by the incubation for 1 hr at room temperature with diluent buffer. The rods are then incubated with diluted antiserum for 16 hr at 4°C, washed 4 times in 0.05% Tween 20 in PBS and incubated with a secondary antibody, (e.g., goat anti-mouse or anti-rabbit IgG) coupled to alkaline phosphatase for 1 hr at room temperature. The presence of the conjugate antibody on the tips can then be detected by reaction with substrate solution.

Syncytia Inhibition Assay A recombinant vaccinia virus syncytium inhibition assay can be used to assess the effectiveness of antisera raised using the MAPS herein described. This assay can be performed using cells infected with a vaccinia virus expressing an HIV *env* gene rather than actual HIV infected cells. Construction of a recombinant vaccinia virus capable of expressing the full-length HIV envelope gene from a vaccinia virus promoter is described in EP Publication No. 0 243 029, hereby incorporated by reference.

Antibody Response to Mono-Epitope MAPS

Nine peptides from the V3 regions of HIV-I isolates III_B, RF and MN were incorporated into tetravalent MAPS (prepared as described above). These MAPS are designated MAP B1 through B9 to indicate that they include a B cell epitope. Referring to Fig. 1, parallel groups of three peptides with chain lengths spanning from 11 to 24 residues were synthesized in MAPS format for each isolate. Tetravalent MAPS were prepared since they have been shown to be as effective as the octavalent MAPS (Tam et al., *J. Exp. Med.* 171:299, 1990). Mice, rabbits and guinea pigs were immunized with one of the nine mono-epitope MAPS. The antisera were analyzed for reactivity against both the immunizing peptide (Fig. 2, panel A) and gp120 (Fig. 2, panel B) in an ELISA assay.

Referring to Fig. 2, panel A, ELISA assays demonstrated that antisera titers in mice were closely related to the length of the III_B peptide used for the immunization, with the MAPS using the longest peptide (B1, amino acids 308-331) inducing the strongest response, followed by the MAPS using the intermediate peptide (B2, amino acids 312-328) which elicited a reduced reactivity, while the MAPS using the shortest peptide (B3, amino acids 315-325) was completely non-immunogenic. The same pattern of ELISA reactivity was observed against native gp120 protein (Fig. 2, panel B). The good response elicted by the B1 MAPS suggests that the B1 peptide, derived from the HIV-I-III_B sequence, contains a T helper cell determinant. Evidently, this epitope is completely lost when the peptide is reduced to only 11 amino acids as in the B3 peptide. This was confirmed by the induction of specific proliferative response in the lymph nodes of mice immunized with the B1 peptide and not with the B3 peptide (described in detail below). The presence of a T cell epitope within this

portion of the V3 loop in a III_B peptide (residues 303-321) was observed by others in goats (Hart et al., *J. Immunol.* 145:2677, 1990). Moreover (Table 1), there was no substantial antibody production in mice against the two other series of peptides, RF (B4-B6) and MN (B7-B9), except for a low reactivity in the group immunized with B8 (MN isolate).

Referring to Table 1, ELISA assay of the rabbit antisera showed that nearly all of the mono-epitope MAPS were able to elicit strong responses; antibody titers varied from the highest value (1.2×10^6) in the antisera of rabbits immunized by the B2 and B8 peptides, to the lowest (2.3×10^4) found in the antisera of rabbits injected with the shortest peptide of the RF series (B6).

Referring to Table 2, the responses of the guinea pigs were uneven. While all MAPS having a peptide derived from the HIV-I-III_B isolate (B1-B3) produced good responses, only the two longer of the sequences of the RF isolate (B4 and B5) and the MN isolate (B7 and B8) were able to elicit a response.

Table 1: Comparison of mouse and rabbit antibody response to mono-and di-epitope MAPS

Mono-epitope MAPS Antisera Titers ($x10^3$)			Di-epitope MAPS Antisera Titers ($x10^3$)		
	Mouse	Rabbit		Mouse	Rabbit
B1 =	125 (± 14)	400 (± 50)	B1T =	390 (±106)	2500 (± 20)
B2 =	85 (± 21)	1225 (±775)	B2T =	500 (±202)	1750 (±500)
B3 =	3 (± 3)	750 (±250)	B3T =	113 (± 80)	1250 (± 50)
B4 =	0	600 (±400)	B4T =	775 (±338)	1250 (±750)
B5 =	0	522 (±477)	B5T =	457 (±251)	725 (±275)
B6 =	7 (± 4)	23 (± 7)	B6T =	782 (±247)	1200 (±400)
B7 =	0	700 (±100)	B7T =	306 (±110)	1020 (±200)
B8 =	40 (± 32)	1200 (±500)	B8T =	143 (± 28)	2100 (±100)
B9 =	0	120 (± 0)	B9T =	22 (± 14)	400 (± 0)

Synthetic MAP peptides containing HIV-I B cell or B cell and T cell epitopes from different HIV-i isolates were used to inoculate animals. The resulting immune sera were assayed by ELISA against the immunizing peptide; the antibody titers are presented as the geometric mean (±SEM) of endpoint dilutions corresponding to antisera from five (mice) or two (rabbits) animals.

Table 2: Comparison of guinea pig antibody response to mono-and di-epitope MAPS

Antibody Titers ($x10^3$)		
IIIB isolate	RF isolate	MN isolate
B1 = 33.6 (± 8.2)	B4 = 3.6 (± 1.9)	B7 = 2.6 (± 0.8)
B2 = 10.1 (± 1.9)	B5 = 3.2 (± 1.6)	B8 = 8.5 (±3.0)
B3 = 16.7 (±10.0)	B6 = -	B9 = -
B1T = 50.1 (± 2.0)	B4T = 31.1 (±36.6)	B7T = ND
B2T = 25.1 (±14.0)	B5T = 1.3 (± 0.4)	B8T = ND
B3T = 17.6 (± 8.1)	B6T = 1.1 (± 0.1)	B9T = ND

ELISA with guinea pig antisera was performed using plates coated with 24 amino acid peptides RP135 (IIIB), RP139 (RF), or RP142 (MN). Titers are the geometric mean (±SEM) of endpoint dilutions of the antisera from three animals.

Antibody Response to Di-Epitope MAPS

Mice, rabbits and guinea pigs were immunized with one of the nine di-epitope MAP constructs. Each di-epitope MAPS contains a tandem configuration in which a T-helper cell peptide derived from HIV-I envelope protein was added at the carboxyl-end of each B cell peptide. Accordingly, these MAPS are referred to as MAP B1T through MAP B9T to indicate that they include a T cell

epitope in addition to one of the B cell epitope peptides, B1-B9. A 16 amino acid peptide, located in the fourth conserved domain of gp120 (III_B isolate, residues 429-443), was selected because it stimulates T helper cell activity in mice (Cease et al., *Proc. Nat'l. Acad. Sci. USA* 84:4249), and probably humans (Berzofsky et al., *Nature* 334:706, 1988) and goats (Palker et al., *J. Immunol.* 142:3612, 1989).

As summarized in Table 1, addition of a T cell peptide in the di-epitope MAPS constructs used for the immunization substantially increased mouse immune response. When mice were injected with the longest di-epitope MAP of the III_B series (B1T MAPS), the immune response was only slightly higher than that induced by the corresponding mono-epitope MAPS (B1 MAPS), probably because a T-helper epitope is present in the B1 sequence. However, a much improved antibody titer was observed in the mice injected with B2T MAPS than in those receiving the intermediate-length mono-epitope B2 MAP. In contrast to the response elicited by B3 MAPS, a very strong antibody induction was observed using B3T MAPS. Similar findings were also observed for RF and MN series MAPS; totally non-immunogenic mono-epitope MAPS, such as B4, B5, B7 and B9, stimulate humoral immune responses in the di-epitope MAPS, B4T, B5T, B7T and B9T.

In rabbits (Table 1), antisera elicited by each of the di-epitope MAPS showed greater immunoreactivity than antisera raised against the corresponding mono-epitope MAP constructions. The enhancement of immune responses in rabbits were less marked than in mice, since in rabbits the mono-epitope MAP constructs alone were able to elicit strong antibody responses, without the use of an additional T-helper epitope.

Referring to Table 2, in guinea pigs, the addition of a T-helper epitope did not consistently enhance the

immune response induced by mono-epitope MAPS in the RF series.

In the one case tested, the comparative immunization of mice with a di-epitope peptide in MAP configuration (MAP-B4T) and the corresponding linear peptide (B4T) revealed that a greater immune response was induced by the tetrameric MAP.

Specificity of the Antibody Response

To characterize the specificity of the B cell response to MAPS B1 and B1T, antisera were assayed for their reactivity to B cell epitope peptides and T cell epitope peptides (Table 3). The mono-epitope B1 MAP induced antisera which reacted with B1 peptide; the di-epitope B1T MAP induced antisera which reacted with the T peptide in addition to the B1 peptide. These results demonstrate that both B and T epitopes are immunogenic and that the T cell epitope also serves as a B cell epitope. Furthermore, these results show that the antisera are specific, since the B1 MAPS induced antisera did not react with the T peptide.

Table 3: Specificity of the Antibody Response to Mono-or Di-Epitope MAPS

	Peptide on plate	Competing Peptide	O.D. (405 nm)
Antisera to B1 MAP	B1	--	1.61
	B1	B1	0.05
	T	--	0.10
	T	B1	0.05
Antisera to B1T MAP	B1	--	1.32
	B1	B1	0.08
	T	--	1.51
	T	B1	1.62

Peptide blocking ELISA assays were performed with a dilution of rabbit antisera giving an absorbance between 1.3 and 1.6. The wells were coated with 5 µg of the indicated peptide. The competing peptide was present at 50 µg/ml.

Cross-Reactivity of the Antibody Response Among HIV Isolates

Rabbit antisera were analyzed for their ability to react with peptides derived from HIV-I isolates other than the ones used to raise them.

Referring to Table 4, there was a moderate level of cross-reactivity between III_B and RF isolates. The antisera to B1 peptide from III_B was able to bind the homologous B4 peptide from RF, although less strongly. Conversely, the B4 antisera reacted with B1 peptide. In contrast, antisera from rabbits immunized with B7 peptide from MN isolate did not react with either B1 or B4 peptides but preferentially only with its own peptide. The cross-reactivity between III_B and RF peptides is likely due to the close homology of the B1 and B4 peptides at their amino-terminus. Seven consecutive amino acids (position 308-314), preceding the conserved central tripeptide GPG, are the same in these two sequences. Indeed, analysis of rabbit antisera raised using MAP-B4 showed a strong cross-reactivity to B1T peptide only over the first 8 amino acids of B1T. In contrast, B7 antisera (MN isolate) was far less reactive with B1 peptide. The B1 and B7 peptides share little homology at the amino-end of each sequence. Thus it appears that the observed cross-reactivity results from recognition of related sequences at the amino terminus of the III_B and RF peptides.

Table 4: Crossreactivity of MAP-induced Antisera[a]

Anti-B1 MAP sera	Anti-B4 MAP sera	Anti-B7 MAP sera
B1 peptide = 350,000	B4 peptide = 570,000	B7 peptide = 660,000
B4 peptide = 30,000	B1 peptide = 41,000	B1 peptide = 25,000
B7 peptide = 4,000	B7 peptide = ---	B4 peptide = ---
gp120-IIIB = 90,000	gp120-IIIB = ---	gp120-IIIB = ---

[a] Rabbits were immunized with the indicated MAP, and the immune sera were analyzed for their ability to bind resin-bound peptides or gp120-III_B. Results are expressed as the geometric mean of several ELISA titers.

Proliferative T Cell Response to B1T and B3T MAPS

A T cell poliferation assay was performed using B1T MAPS and B3T MAPS. Lymph node cells from antigen-primed Balb/c mice were cultured *in vitro* with the same BT MAPS used for the immunization or with the corresponding B cell epitope MAPS. As shown in Table 5, lymph node cells from mice immunized with B1T MAPS proliferated when cultured with B1 MAPS (which appears to have a helper deteminant) and even more strongly when the BIT MAPS was used for the *in vitro* stimulation. Further, the B3T MAPS-primed cells exhibited a high level of proliferation following stimulation with the di-epitope B3T MAPS. In contrast, the B3 peptide did not induce proliferation.

Table 5: Proliferative T-cell Response Induced by B1T MAPS and B3T MAPS

T cell primed with[a]	Restimulated with[b]	^{3}H-Thymicine incorporation	Stimulation Index
B1T MAPS	---	3.2 ± 0.2	1.0
	B1 MAPS	17.7 ± 0.6	4.5
	B1T MAPS	33.4 ± 1.1	8.4
B3T MAPS	---	3.6 ± 0.2	1.0
	B3 MAPS	4.1 ± 0.3	1.1
	B3T MAPS	38.2 ± 2.8	10.4

[a] Mice (3 Balb/c per group) were injected with 50µg MAPS in Fruend's complete adjuvant.
[b] Restimulated with 10µg/ml MAPS in virto.

Biological Activity of the Immune Response

The biological activity of the rabbit and guinea pig MAPS induced antisera were tested in a syncytium inhibition assay, utilizing CEM cells infected with a recombinant vaccinia virus expressing gp160 of the III_B, RF or MN strain. Titers obtained in such an assay generally correlate with the ability of sera to neutralize cell free virus *in vitro*. The results of this assay are presented in Tables 6 and 7 which lists the reciprocal of the antiserum dilution which reduces the number of syncytia formed by 90%.

Table 6: MAPS-Induced Rabbit antisera syncytia inhibition titers against different HIV-I isolates[a]

III_B	RF	MN
B1 = 20, <20	B4 = 40, 40	B7 = 160, 320
B2 = <20, 40	B5 = 80, <20	B8 = 640, 640
B3 = 40, <20	B6 = <20, <20	B9 = 160, <20
B1T = 320, 80	B4T = 160, 160	B7T = 320, 320
B2T = <20, 40	B5T = 40, 40	B8T = 160, 640
B3T = <20, <20	B6T = 160, 80	B9T = <20, 40

[a] Rabbit immune sera were tested for their ability to inhibit HIV-I-1 envelope protein-induced syncytium formation. Fusion inhibition titer is the reciprocal of the dilution that reduces the number of syncytia by 90%. Serum from two rabbits in each group was analyzed.

Table 6 demonstrates that the majority of the rabbits developed antisera that inhibited syncytia formation in a culture expressing envelop of the same strain used as an immunogen. The data suggest that inhibitory antibodies are elicited primarily by the di-epitope MAPS for the RF series and to some extent for the III_B series. In rabbits, the T cell epitope does not seem to influence the response of MAPS in the MN series which appears to be significantly greater than the response elicited by MAPS in the III_B and RF series. For instance, in the III_B series, B1T MAP induced an antisera which was better at inhibiting syncytia formation than B1 MAP. A clear correlation between the titer observed in ELISA assay and the syncytium inhibition activity of the antibodies was not evident, since antisera which show very high peptide binding capacity were less effective than others in inhibiting syncytium formation.

Table 7 presents the results of similar syncytium inhibition assays in guinea pigs. These data show that MAPS in the MN series elicit a better immune response than MAPS in the RF or III_B series and that the B8 peptide appears to be the most effective peptide in the MN series.

Table 7: MAPS-Induced Guinea Pig antisera syncytia inhibition titers against different HIV-I isolates[a]

III_B	RF	MN
B1 = 20, <20, 40	B4 = 20, <20, <20	B7 = 320, >640, 320
B2 = <20, <20, <20	B5 = 20, <20, <20	B8 = 1280, 1280, 1280
B3 = <20, <20, <20	B6 = 20, <20, <20	B9 = <20, <20, <20
B1T = 80, 80, 80	B4T = 320, <20, <20	B7T = ND
B2T = <20, <20, <20	B5T = 80, 80, 40	B8T = ND
B3T = <20, <20, <20	B6T = 40, <20, <20	B9T = ND

[a] Guinea pig immune sera were tested for their ability to inhibit HIV-I-1 envelope protein-induced syncytium formation. Fusion inhibition titer is the reciprocal of the dilution that reduces the number of syncytia by 90%. Serum from three guinea pigs in each group was analyzed.

Therapy

The MAPS of the invention may be administered by direct injection into the blood stream. They may also be incorporated into polymeric microcapsules (e.g., liposomes) and/or administered with an adjuvant (e.g., alum). Liposome-encapsulated antigens often elicited a higher antibody titer than non-encapsulated antigens.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANTS: REPLIGEN CORPORATION
THE ROCKEFELLER UNIVERSITY

(ii) TITLE OF INVENTION: MULTIPLE ANTIGEN PEPTIDES FOR USE AS HIV VACCINES

(iii) NUMBER OF SEQUENCES: 21

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Fish & Richardson
(B) STREET: 225 Franklin Street
(C) CITY: Boston
(D) STATE: Massachusetts
(E) COUNTRY: U.S.A.
(F) ZIP: 02110-2804

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5" Diskette, 1.44 Mb
(B) COMPUTER: IBM PS/2 Model 50Z or 55SX
(C) OPERATING SYSTEM: IBM P.C. DOS (Version 3.30)
(D) SOFTWARE: WordPerfect (Version 5.1)

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 744,281
(B) FILING DATE: 13 August 1992

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Paul T. Clark
(B) REGISTRATION NUMBER: 30,162
(C) REFERENCE/DOCKET NUMBER: 00231/052WO1

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (617) 542-5070
(B) TELEFAX: (617) 542-8906
(C) TELEX: 200154

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Cys Arg Ile Lys Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala
5 10 15

Met Tyr Ala Pro Pro Ile Ser Gly Gln Ile Arg Cys
20 25

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Gln Ser Val Glu Ile Asn Cys Arg Thr Pro Asn Asn Asn Thr Arg Lys
5 10 15

Ser Ile Arg Ile Gln Arg Gly Pro Gly Arg Ala Phe Val Thr Ile Gly
20 25 30

Lys

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Ile Gly Pro Gly Arg
5

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Lys Arg Lys Arg Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr
5 10 15

Lys

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala
5 10 15

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

His Ile Gly Pro Gly Arg
5

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Ile His Ile Gly Pro Gly Arg
5

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Arg Ile His Ile Gly Pro Gly Arg
5

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Ile Gly Pro Gly Arg Ala
5

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Ile Gly Pro Gly Arg Ala Phe
5

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Lys Arg Lys Arg Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr
5 10 15

Lys Asn

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Asn Asn Thr Arg Lys Ser Ile Arg Ile Gln Arg Gly Pro Gly Arg Ala
5 10 15

Phe Val Thr Ile Gly Lys Ile Gly
20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Lys Ser Ile Arg Ile Gln Arg Gly Pro Gly Arg Ala Phe Val Thr Ile
5 10 15

Gly

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Arg Ile Gln Arg Gly Pro Gly Arg Ala Phe Val
5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Asn Asn Thr Arg Lys Ser Ile Thr Lys Gly Pro Gly Arg Val Ile Tyr
5 10 15

Ala Thr Gly Gln Ile Ile Gly
20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Thr Arg Lys Ser Ile Thr Lys Gly Pro Gly Arg Val Ile Tyr Ala Thr
5 10 15

Gly

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Ser Ile Thr Lys Gly Pro Gly Arg Val Ile Tyr
5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Pro Asn Tyr Asn Lys Arg Lys Arg Ile His Ile Gly Pro Gly Arg Ala
5 10 15

Phe Tyr Thr Thr Lys Asn Ile Ile
20

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Lys Arg Lys Arg Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr Thr
5 10 15

Lys Asn

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Arg Ile His Ile Gly Pro Gly Arg Ala Phe Tyr Thr
5 10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

Gln Ile Ile Asn Met Trp Gln Glu Val Gly Lys Ala Met Tyr Ala
5 10 15

Claims

1. A multiple antigen peptide system comprising a dendritic core covalently attached to a peptide, said peptide including the sequence IGPGR (SEQ ID NO: 3), said multiple antigen peptide system, when injected into a mammal, being capable of eliciting an immune response.

2. The multiple antigen peptide system of claim 1 wherein said peptide includes a pair of six amino acid sequences flanking said sequence IGPGR (SEQ ID NO: 3), said flanking sequences taken together having at least 36% homology to the pair of six amino acid sequences flanking the sequence IGPGR within the V3 loop of HIV-I-MN prototype virus.

3. The multiple antigen peptide system of claim 1 wherein said peptide includes the sequence KRKRIHIGPGRAFYTTK (SEQ ID NO: 4).

4. The multiple antigen peptide system of claim 1 further comprising a covalently attached T cell epitope.

5. The multiple antigen peptide system of claim 4 wherein said T cell epitope is covalently linked in tandem to said peptide.

6. The multiple antigen peptide system of claim 4 wherein said T cell epitope includes the sequence QIINMWQEVGKAMYA (SEQ ID NO: 5).

7. The multiple antigen peptide system of claim 1 wherein said dendritic core includes lysine.

8. The multiple antigen peptide system of claim 1 wherein said dendritic core is tetravalent.

9. The multiple antigen peptide system of claim 1 wherein said peptide is between 10 and 40 amino acids long.

10. The multiple peptide antigen system of claim 1 wherein said peptide includes the sequence HIGPGR (SEQ ID NO: 6).

11. The multiple antigen peptide system of claim 10 wherein said peptide includes the sequence IHIGPGR (SEQ ID NO: 7).

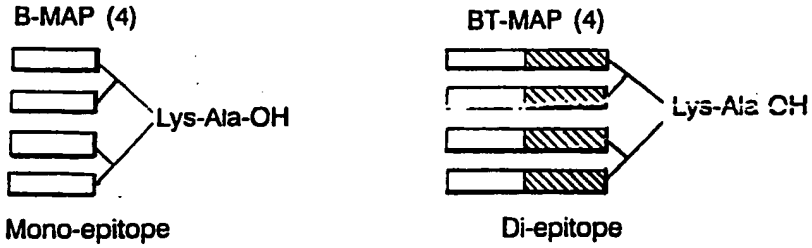
12. The multiple antigen peptide system of claim 11 wherein said peptide includes the sequence RIHIGPGR (SEQ ID NO: 8).

13. The multiple antigen peptide system of claim 12 wherein said peptide includes the sequence IGPGRA (SEQ ID NO: 9).

14. The multiple antigen peptide system of claim 13 wherein said V3 loop peptide includes the sequence IGPGRAF (SEQ ID NO: 10).

15. The multiple antigen peptide system of claim 14 wherein said V3 loop peptide includes the sequence KRKRIHIGPGRAFYTTKN (SEQ ID NO: 11).

16. A vaccine comprising an immunologically effective amount of the multiple antigen peptide system of claim 1.



B1	308 NNTRKSIRIQRGPGRAFVTIGKIG 331	(SEQ ID NO: 12)	
B2	312 KSIRIQRGPGRAFVTIG 328	(SEQ ID NO: 13)	IIIB isolate
B3	315 RIQRGPGRAFV 325	(SEQ ID NO: 14)	
B4	322 NNTRKSITKGPGRVIYATGQIIG 344	(SEQ ID NO: 15)	
B5	324 TRKSITKGPGRVIYATG 340	(SEQ ID NO: 16)	RF isolate
B6	327 SITKGPGRVIY 337	(SEQ ID NO: 17)	
B7	306 PNYNKRKRIHIGPGRAFYTTKNII 329	(SEQ ID NO: 18)	
B8	310 KRKRIHIGPGRAFYTTKN 327	(SEQ ID NO: 19)	MN isolate
B9	313 RIHIGPGRAFYT 324	(SEQ ID NO: 20)	
T	429 QIINMWQEVGKAMYA 443	(SEQ ID NO: 21)	IIIB isolate

FIG. 1

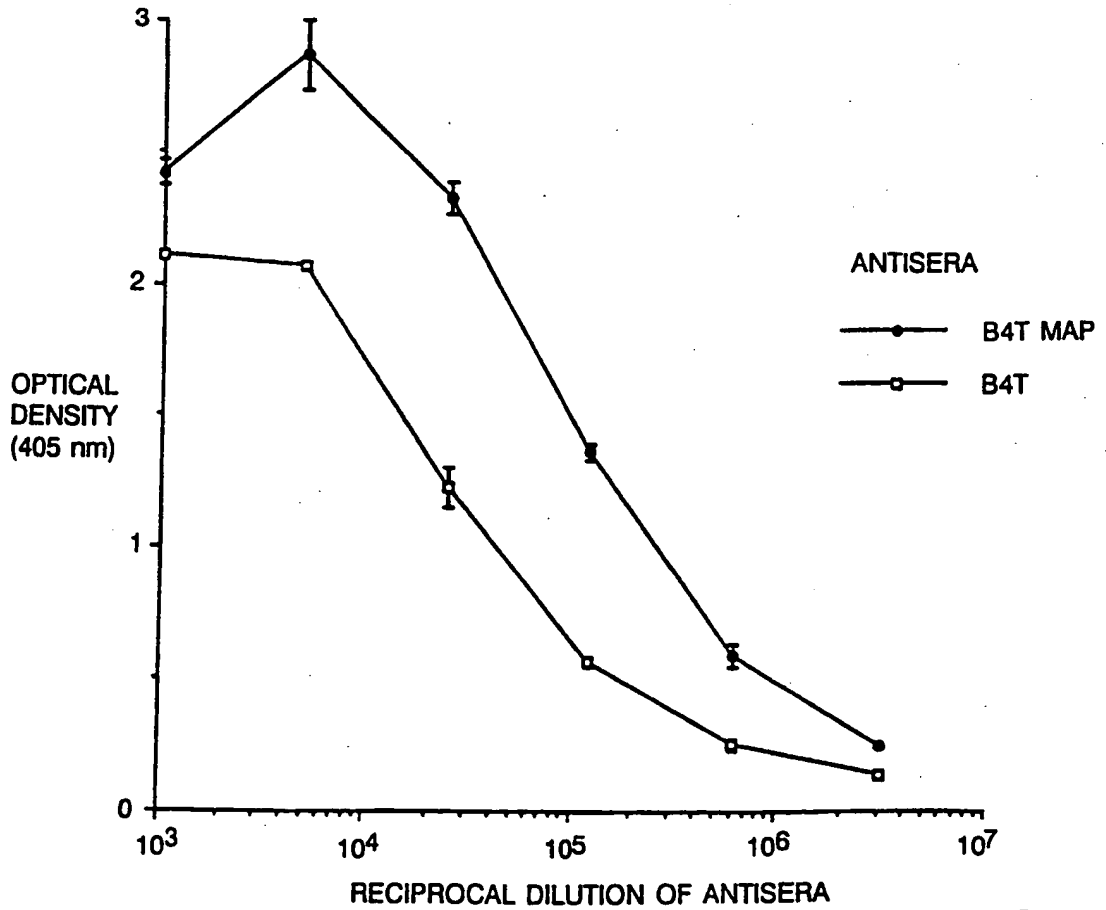


FIG. 3

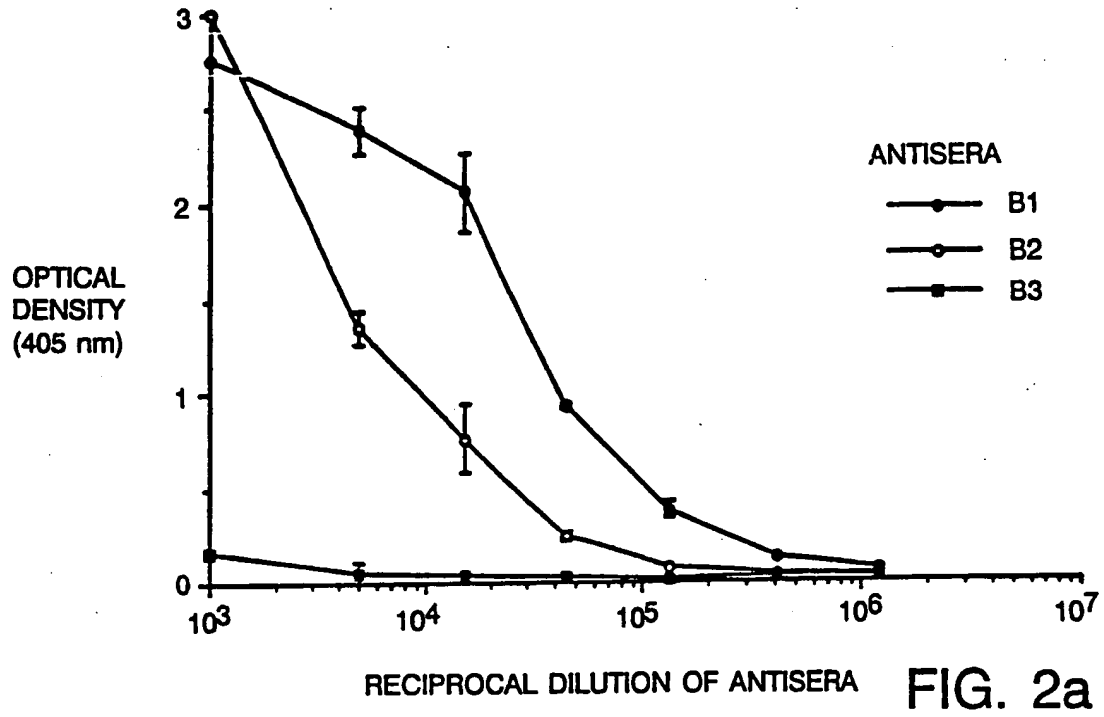

OPTICAL DENSITY (405 nm)
3
2
1
0
ANTISERA
B1
B2
B3
10^3
10^4
10^5
10^6
10^7
RECIPROCAL DILUTION OF ANTISERA

FIG. 2a

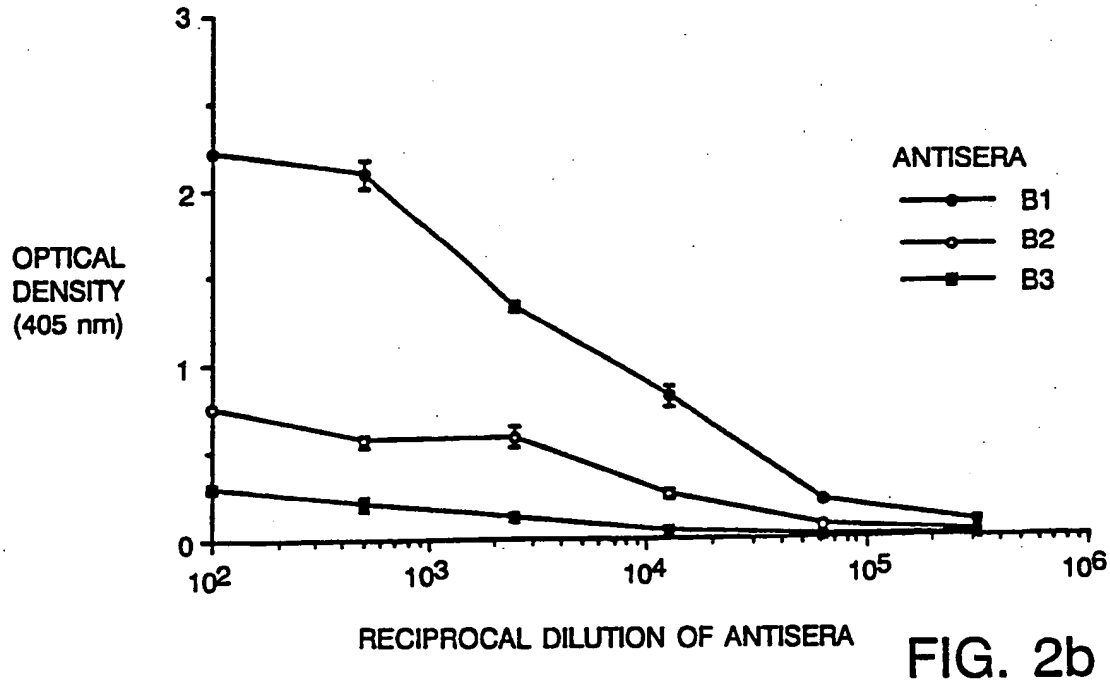

OPTICAL DENSITY (405 nm)
3
2
1
0
ANTISERA
B1
B2
B3
10^2
10^3
10^4
10^5
10^6
RECIPROCAL DILUTION OF ANTISERA

FIG. 2b

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/89, 88; 530/317, 321, 324, 325, 326, 327, 328, 329, 330, 345, 395, 403, 405, 409, 806, 807; 435/961, 974; 436/823; 930/221

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS online, file Registry, IntelliGenetics, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	EP, A, 0,328,403 (Wang) 16 August 1989, see pages 2, 5 (lines 54-65), 6 (lines 44-64), 8 (Table IIb, lines 45-55), 9 (lines 29-32), 13, 14, claims 1, 4, 6, 7.	1, 2, 4, 6, 7, 9, 13, 14, 16 3, 5, 10-12, 15
Y	The Journal of Immunology, Vol. 142, No. 10, issued 15 May 1989, T. J. Palker et al, "Polyvalent human immunodeficiency virus synthetic immunogen comprised of envelope gp120 T helper cell sites and B cell neutralization epitopes", pages 3612-3619, see pages 3612-3614.	1, 2, 4-7, 9-13, 16
Y	Proc. Natl. Acad. Sci. USA, Vol. 87, issued November 1990, C. F. Scott, Jr. et al, "Human monoclonal antibody that recognizes the V3 region of human immunodeficiency virus gp120 and neutralizes the human T-lymphotropic virus type III_{MN} strain", pages 8597-8601, see pages 8597, 8598.	1-16
Y	The Journal of Biological Chemistry, Vol. 263, No. 4, issued February 1988, D. N. Posnett et al, "A novel method for producing anti-peptide antibodies", pages 1719-1725, see pages 1720-1721.	1-16

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
22 October 1992	NOV 1992
Name and mailing address of the ISA/ Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer KAY K. KIM, PH.D.

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP, A, 0,339,695 (Bloemhoff et al) 02 November 1989, see pages 2 (lines 11-18, 54), 3 (line 1), 4 (line 56) to 5 (line 5).	1-7, 9-16
Y	Journal of Experimental Medicine, Vol. 171, issued January 1990, J. P. Tam et al, "Incorporation of T and B epitopes of the circumsporozoite protein in a chemically defined synthetic vaccine against malaria", pages 299-306, see page 303.	4, 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/06688

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (5):

A61K 39/385, 39/21, 39/12, 47/48; CO7K 17/02, 7/02, 7/06, 7/08, 7/10

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/89, 88; 530/317, 321, 324, 325, 326, 327, 328, 329, 330, 345, 395, 403, 405, 409